Abstract

Charles University in Prague, Faculty of Pharmacy in Hradec Králové
Department of Analytical Chemistry
Candidate: Kateřina Štětková
Supervisor: RNDr. Hana Vlčková, Ph.D.
Title of Diploma thesis: Study of influence of mobile phase composition on selectivity and retention of the analytes on HILIC stationary phases

This graduation thesis deals with an influence of concentration of ACN, pH and buffer concentration in mobile phase on retention of selected analytes and on selectivity of Atlantis HILIC Silica column (3 μm, 2,1 mm x 100 mm) and Luna NH_2 column (3 μm, 2,0 mm x 100 mm). A set of 35 analytes including neutral, basic and acidic compounds were chosen for this study. Detection was performed by PDA detector at the wave-length of 245 nm. Measurements were carried out using isocratic flow 0.4 $ml.min^{-1}$ of mobile phase ACN/water (buffer). The column temperature was set up to 30 °C, auto sampler temperature to 4 °C and injection volume to 2 μl. For easier evaluation of results, measured data are presented in charts and tables. These were used to evaluation of columns in terms of selectivity and retention of the individual groups of analytes.

The typical HILIC behavior, an increasing retention of analytes with increasing ACN concentration in mobile phase, was observed for Atlantis HILIC Silica column. The most intensive effect was shown at more than 85% of ACN. Luna NH_2 column except for HILIC behavior shown an extremely strong retention of acidic compounds in RP mode. Absolutely opposite effect was observed with increasing concentration of buffer in mobile phase, while retention of basic compounds was decreasing and retention of acidic compounds was increasing on Atlantis HILIC Silica column, the retention of basic compounds was increasing and retention of acidic compounds was decreasing on Luna NH_2 column. A change of buffer concentration had an insignificant effect on retention of neutral compounds and nucleotide bases. The differences in selectivity of Atlantis HILIC Silica and Luna NH_2 were more significant at pH = 3.8 than pH = 6.8. The most significant differences in selectivity were shown for acidic compounds.